# VOLUMETRIC CHANGES IN THE EGG OF THE BROOK LAMPREY, ENTOSPHENUS (LAMPETRA) WILDERI (GAGE), AFTER FERTILIZATION.[1]

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Various investigators have found by actual measurements that the eggs of certain animals do not change appreciably in volume as a result of fertilization. Calberla[2] in his work on the fertilization process in the egg of *Petromyzon planeri* says: "Genaue Messungen mittelst des Zeichnenprismas (Oberhäuser), die während des Befruchtungsvorganges angestellt wurden, erwiesen es als zweifellos, dass der Dotter sich nicht contrahirt, dagegen die Eihaut sich enorm ausdehnt. . . . Allerdings nimmt der Dotter, nachdem er von der Eihautberührung befreit worden ist, eine andere Form an, d. h. er geht von der Ellipsoidform in die Kugelform über, aber dies geschieht ohne Volumsverminderung." McClendon,[3] working on the eggs of *Arbacia punctulata*, found that when the eggs were placed in a molecular solution of cane sugar, approximately isosmotic with sea water, the mean diameter of a certain number of eggs was 83 when not fertilized and 75 when fertilized. Control eggs in ordinary sea water showed mean diameters of 83 and 86 respectively when unfertilized and fertilized. According to these results the eggs of *Arbacia punctulata* do not decrease in size as a result of fertilization under normal conditions but become larger instead. Loeb[4] found that the volume of the egg of *Strongylocentrotus* does not change appreciably as a result of fertilization. He says: "Ich habe eine Reihe von Messungen der Durchmessers des Cytoplasmas vor und nach der Befruchtung mit Hilfe des Zeichenapparates ausgeführt, wohin es sich herausstellte, dass das Volumen des Eicytoplasmas von *Strongylocentrotus* bei der

[1] Contribution from the Zoölogical Laboratory of the University of Michigan, No. 141.

[2] Calberla, Ernst, *Zeitschr. f. wiss. Zool.*, Vol. 30, 1877.

[3] McClendon, J. F., *Science*, N.S., Vol. 32, 1910.

[4] Loeb, J., *Arch. f. Entwicklungsmechanik*, Vol. 26, 1908.

Membranbildung keine mit dieser Methode wahrnehmbare Volumänderung erfährt. Das beweist mit Sicherheit, dass der Inhalt des Membranraums hauptsächlich Seewasser ist, das vom aussen in demselben diffundiert."

In some work that I have done[1] on the fertilization process in the brook lamprey, *Entosphenus wilderi* (Gage), I have obtained

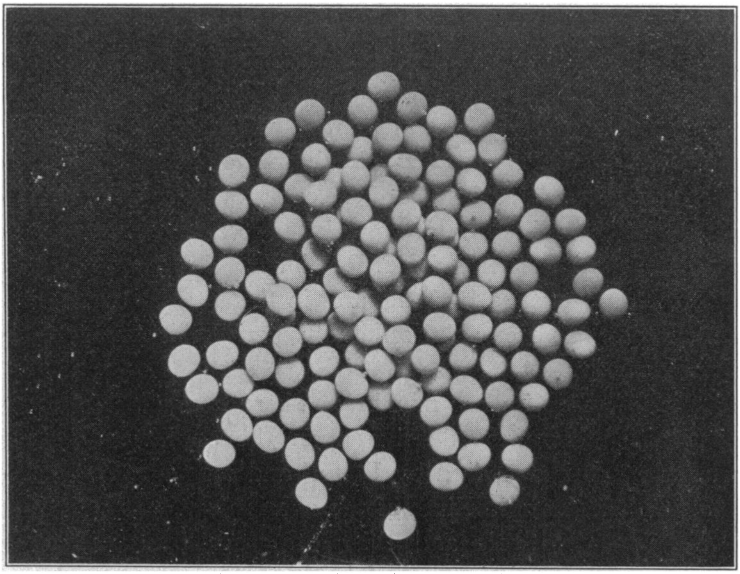

FIG. 1. Unfertilized eggs of *Entosphenus wilderi*.

results which indicate that the eggs in this form decrease considerably in size after fertilization.

Herfort[2] has given a careful description of the egg of *Petromyzon fluviatilis* and I have verified most of his observations on the egg of the American brook lamprey. Since there is no essential difference between the eggs of the two species, only a brief description is necessary here. Before fertilization the egg approaches very closely to an ellipsoid of revolution in shape although it is slightly more pointed at the animal pole (Fig. 1). It

[1] Presented to the Michigan Academy of Science at its nineteenth annual meeting in Ann Arbor, April 3, 1913. Fifteenth Report of the Michigan Academy of Science.

[2] Herfort, Karl, *Arch. f. mikr. Anat.*, Vol. 57, 1901.

possesses a distinct membrane which has been termed by Lubosch[1] and others the zona radiata but to which I shall apply the more general term vitelline membrane. In the ovarian egg of the larva this membrane consists of a single layer but after metamorphosis it becomes differentiated into two distinct layers which increase in thickness as the egg approaches maturity. The outer layer usually shows faint striations which have been supposed to represent canals. The two layers are very firmly united. Beneath the vitelline membrane there is a cortical alveolar layer which is covered on the outside by a very thin protoplasmic layer (vitelline membrane of Lubosch). This thin protoplasmic layer adheres very closely to the vitelline membrane and is continuous with the rest of the egg protoplasm through the walls of the alveoli. The alveolar layer seems to be similar to that in *Nereis* as described by F. R. Lillie.[2] The same condition has also been described by Reighard[3] in the egg of the wall-eyed pike. I have not been able to find any perivitelline space in the unfertilized egg.

Outside of the vitelline membrane there is a gelatinous substance which becomes adhesive upon the addition of water. This substance is supposed by Böhm[4] and others to be derived from the follicular epithelium of the ovarian egg. It is thickest at the vegetative pole and thins off gradually towards the animal pole. Here it is covered by a mucous mass which was first described by A. Müller[5] who called it the "Flocke." It is in the "Flocke" that most of the spermatozoa are found after the egg is fertilized. The vitelline membrane is slightly modified at the animal pole to form a region permeable to the spermatozoa but there appears to be no true micropyle present.

After fertilization visible changes almost immediately take place which result in the separation of the vitelline membrane from the egg and the formation of a large perivitelline space (Fig. 2). This separation is initiated a short distance from the middle of the animal pole as an indentation of the cortical layer, and a

[1] Lubosch, W., *Jen. Zeitschr. f. Naturwiss.*, Vol. 38, 1904.

[2] Lillie, F. R., *Jour. Morph.*, Vol. 22, 1911.

[3] Reighard, Jacob, Tenth Biennial Report of the State Board of Fish Commissioners (Michigan), 1893.

[4] Böhm, A. A., *Arch. f. Mikr. Anat.*, Vol. 32, 1888.

[5] Müller, A., Herrn Karl von Baer zur Feier des 50. Jahrestages, u.s.w., 1864.

circular perivitelline space is formed which extends around the pole in the form of a groove. This groove widens rapidly towards the animal pole until it forms a large space separating the egg at this pole from the vitelline membrane. At the same time a wave of contraction passes over the egg towards the vegetative pole and within a few minutes the whole egg is separated from the membrane. During the contractions of the egg, the long and short diameters vary considerably from time to time. I have

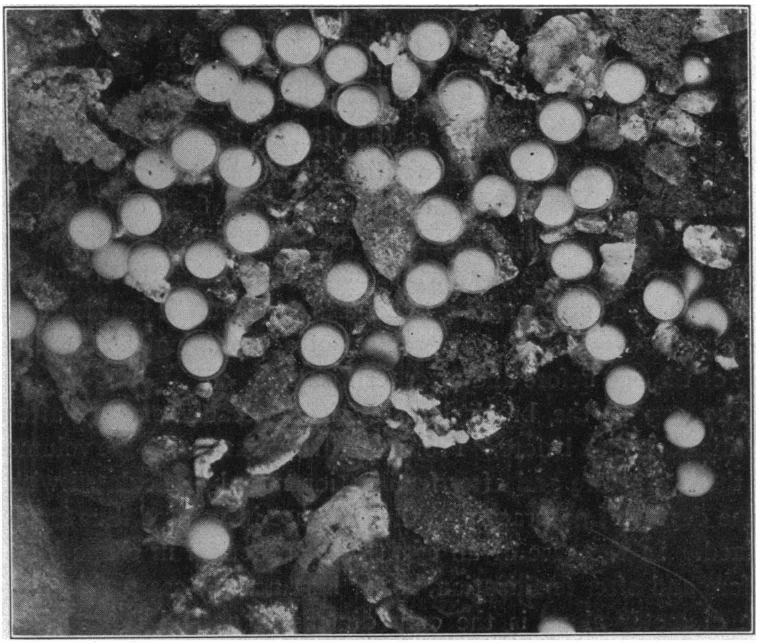

FIG. 2. Fertilized eggs of *Entosphenus wilderi*. ×6.

made accurate measurements at short intervals which show that the contractions are real and not merely apparent. They are very striking and remind one of peristaltic waves or ameboid movements.

The whole process during which the membrane separates from the egg lasts about five minutes and when the egg comes to rest again it has assumed a spherical shape instead of the previous ellipsoidal or ovoidal shape. During the process the vitelline membrane becomes greatly distended. When the membrane

first begins to separate from the egg, the walls of the alveoli of the cortical layer are drawn out into long slender threads which soon break, the outer part adhering to the vitelline membrane and the inner part fusing with the cytoplasm of the surface of the egg. The contents of the alveoli do not seem to diffuse through the vitelline membrane but remain in the perivitelline space. The thin layer of cytoplasm which adheres to the vitelline membrane seems to be similar to the same layer found in the fertilized egg of *Nereis*. In this form F. R. Lillie regards it as a plasma membrane "comparable in some respects to the fertilization membrane of the sea urchin" (loc. cit., page 364). This thin film does not seem to be a permanent structure in the lamprey egg and probably breaks up as the vitelline membrane distends. A part of it, at least, together with the cytoplasm of the alveolar walls seems to collect into little drops which float off into the fluid of the perivitelline space and eventually disintegrate.

Without going into any further detail concerning the structure of the egg and the changes it undergoes as a result of fertilization, we may now return to our problem of determining the relative size of the egg before and after fertilization.

The egg of the brook lamprey is noticeably smaller after fertilization than before. From careful measurements the volume of the egg before and after fertilization may be calculated with some degree of accuracy and the decrease in volume thus ascertained. In the case of an egg that is spherical in shape both before and after fertilization, as for instance the sea urchin egg, the diameter alone is the only dimension needed in order to calculate the volume. For the unfertilized lamprey eggs a different method must be employed. For purposes of calculation they were considered as perfect ellipsoids of revolution. This introduces an error but I am convinced it can not be very great, as the eggs are very nearly ellipsoidal in shape.

The unfertilized eggs were placed in a watch glass containing ordinary tap water at a temperature of about 19 degrees Centigrade and outlined by means of a camera lucida at a magnification of 80 diameters. These outlines were as nearly as possible optical sections through the plane of the major axis of the eggs and did not include the vitelline membrane. The eggs were then

fertilized and after about ten minutes they were outlined again in a similar manner through the planes of their diameters. The drawings thus obtained were then measured by means of a planimeter which recorded the area in square centimeters. From these areas and the long diameters in the case of the unfertilized eggs, and the areas and the diameters in case of the fertilized eggs, the volumes were calculated according to formulas as follows: The volume of an ellipsoid of revolution is equal to the constant .8488 times the square of the area of the plane of its major axis divided by the major axis; and the volume of a sphere is equal to the constant .8488 times the square of the area of its great circle divided by the diameter.[1] Formulas involving the use of planimeter areas and diameters were used because they seemed to introduce the least number of factors and thus eliminate chances of error. Since the same factors were used in each case the errors ought to average up about the same for both sets of drawings.

The volumes of 48 eggs at a magnification of 80 diameters were thus ascertained before and after fertilization. The volumes thus obtained were divided by the cube of 80 in order to get the actual volumes of the eggs. This gave as a result an average volume of .6017 cubic millimeter for the unfertilized eggs and an average volume of .5205 cubic millimeter for the fertilized eggs. This shows that the average decrease in size is .0812 cubic millimeter or 13.48 per cent. of the original volume.[2]

The above results are so striking that they have seemed worth recording at this time. Analogous conditions have been found by O. C. Glaser[3] in the egg of *Asterias forbesii*. Here the egg

[1] I am indebted to Professor Theodore R. Running for suggesting the use of these formulas.

[2] Professor Reighard has called my attention to the fact that since the unfertilized eggs are heavier than the water in which they are kept and rest on a plane surface, they may be slightly flattened by gravity. The fertilized eggs, on the other hand, which are immersed in a perivitelline fluid of greater density than water and rest on the curved vitelline membrane, are less likely to be flattened by gravity. For these reasons my measurements for the unfertilized eggs might be larger than those for the fertilized. I have examined unfertilized eggs that have been fixed in various fixing solutions but have found no evidence of flattening in these. There is no fresh material available at present. Since the eggs are small and were kept in water when they were outlined it does not seem probable that they would flatten to any appreciable extent.

[3] Glaser, O. C., *Science*, N.S., Vol. 38, 1913.

separates after fertilization from the inner surface of a thin preëxisting membrane and the change does not seem to be due to changes in the fertilization membrane but to changes in the surface film of the egg. The egg of *Asterias* after separating from the membrane was found to be smaller than before. In case of the lamprey egg also the initial change seems to be in the cortical layer of the egg and not in the membrane.

I have succeeded in inducing the egg of the lamprey to go through changes similar to those it exhibits after fertilization, by subjecting it to artificial stimuli, such as shaking, pricking with a needle, exposing to sudden changes of temperature, etc. The membrane separates in a similar manner f om the egg, a peristaltic wave passes over it and it becomes spherical in shape. This indicates that the stimulus that starts the egg on a career of development need not be of any specific kind. Some of the lamprey eggs that have thus been stimulated have segmented parthenogenetically. Bataillon[1] has succeeded in inducing parthenogenetic development of the egg of *Petromyzon planeri* by introducing them into a solution of cane sugar, and he has demonstrated that the segmentation is real and not merely a fragmentation of the egg.

Most of the material that the eggs lose as a result of fertilization is probably water, but it seems to be certain that other substances are also given off. Reighard (loc. cit., page 105) called the fluid contents of the alveoli of the cortical layer in the egg of the wall-eyed pike "cortical drops" and came to the conclusion that these drops consisted of an albuminous substance and that this substance is in solution in the perivitelline fluid. F. R. Lillie (loc. cit., page 365) thinks that the contents of the cortical alveoli in the egg of *Nereis* are unquestionably colloidal. Similar conclusions have been reached by others in other forms. In the lamprey egg after fertilization much of the substance that fills up the space between the egg and the membrane is water that has diffused through from the outside. Some of the substance, however, came from the cortical alveoli and is probably a colloid. F. R. Lillie[2] has found that the unfertilized ova of *Nereis* and *Arbacia* in sea water secrete

[1] Bataillon, E., *Arch. f. Entwicklungsmechanik*, Vol. 18, 1904.

[2] Lillie, F. R., *Science*, N.S., Vol. 38, 1913.

substances which cause agglutination of their respective spermatozoa. He has termed this substance "fertilizin" and assumes that "the union of this substance with the spermatozoa enters in some significant way into the process of development," and that it is located in the cortex of the egg. It may be that the "Flocke" described for the lamprey egg has some significance in this connection for it is in it that the spermatozoa seem to collect in great numbers after fertilization.

Evidences presented in this paper show clearly that the egg of the brook lamprey decreases considerably in size after fertilization. This is contrary to the results obtained by Calberla in the egg of *Petromyzon planeri*, by McClendon in *Arbacia punctulata*, and by Loeb in *Strongylocentrotus*. It harmonizes, however, with the results obtained by Glaser in *Asterias forbesii*. There is reason for believing that some of the substance given off by the egg after fertilization is colloidal in nature. Much of this substance comes from the cortical alveoli.

I desire to express my thanks to Professor Jacob Reighard for his criticism in connection with the preparation of this paper.

ANN ARBOR, MICH.,
November 17, 1913.